

The University of Chicago

CHEMICAL STUDY OF THE
RIPENING PROCESS OF BOSCH PEARS

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY

DEPARTMENT OF BOTANY

1937

By
WILLIAM E. MARTIN

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CHEMICAL STUDY OF THE RIPENING PROCESS OF BOSCH PEARS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 481

WILLIAM E. MARTIN

(WITH FIVE FIGURES)

Introduction

In the commercial production and marketing of pears the fruit is not allowed to ripen on the tree, but is picked while it is still hard and green. Winter varieties are usually placed in storage immediately after picking and packing, and are not marketed until later in the season. Some varieties ripen slowly at storage temperatures while others remain hard and green for long periods and do not ripen until removed from storage and kept for a time at a higher temperature. The Bosch pear is one of the latter class, and tests have shown (9) that ripening temperatures of 65°–70° F. are essential for the development of best eating quality of the fruit.

The process of ripening or conditioning the fruit at these latter temperatures has become general in recent years, and much of the fruit of this variety is brought to the condition known as "firm eating ripe" before it is offered on the retail market.

The physiology of the ripening of summer pears such as the Bartlett has been investigated by several workers (1, 6, 12). There has been comparatively little work done on the chemical changes involved in the stages of ripening of winter pears taking place before the fruit is placed on the market, and no work at all, to the writer's knowledge, on the changes taking place after the fruit has attained optimum eating quality.

In the present investigation the attempt was made to determine chemically the nature of the metabolic changes taking place during the ripening of Bosch pears at 67° F. after removal from storage. The experiments were planned to determine the nature of the ripening process after the point of optimum eating quality had been reached and during the time the fruit was becoming overripe and developing



"internal breakdown." This breakdown, known also as core rot, is characterized by a brown discoloration and softening of the tissues about the core. It appears first at the stem end of the fruit along the vascular elements leading to the core and later develops in the pith parenchyma surrounding the carpels. HARLEY and FISHER (7) have demonstrated that this breakdown of the pear is associated with an accumulation of acetaldehyde in the tissues, and have suggested that after a certain concentration of acetaldehyde is reached browning and breakdown take place.

Bosc pears taste very much sweeter at the time of optimum eating quality than they do when either green or overripe. It was planned, therefore, to determine accurately whether there was any appreciable increase in sugars during ripening or whether the observed increase in sweetness was merely caused by a concentration of solutes as the fruit lost water by transpiration.

The experiment was carried out with three different lots of fruit, one picked at the time of commercial harvest, another ten days earlier, and the third ten days after the commercial crop. A number of representative samples which had received the same treatment as the commercial crop were placed in a ripening room at 67° F., and samples were withdrawn for analysis on each of twenty successive days. At the time the fruit was placed in the ripening room the pears of all three pickings were still hard. The green ground color was beginning to fade and patches of yellow were appearing in the fruit of the late picking.

Material and methods

The fruit used in this investigation was obtained through the courtesy of F. C. REIMER of the Southern Oregon Experiment Station, Talent, Oregon, and was picked from a single Bosc tree.

Three pickings of four boxes each were made on September 3, 13, and 23, 1935. The picking on September 13 corresponded with the commercial harvest of Bosc pears in the region; the other pickings were respectively earlier and later. The maturity of the fruits was measured by the Oregon pressure tester (13) and the results of these measurements are listed in table 1. The pressure test is based on the fact that during the growth and ripening of the pear there is a

gradual and consistent lowering of the physical resistance to pressure or wounding of the epidermal and cortical regions of the fruit.

Immediately after picking, the fruit was wrapped and packed in boxes and placed in storage at 30° – 31° F., and on November 16 it was removed from storage and shipped to Chicago under ventilation, arriving November 25. After arrival, the fruit was removed immediately from the shipping boxes and graded on the basis of size. All fruits injured by stem punctures or damaged by insects were discarded.

The fruit of each picking was then divided into twenty representative lots containing an equal number of large, medium, and small pears. In series A there were twenty pears in each of the twenty

TABLE 1
MATURITY OF PEARS AT TIME OF PICKING

SERIES	PICKED	PRESSURE IN POUNDS
A.....	September 3	24.8
B.....	September 13	21.3
C.....	September 23	17.9

sample lots put up for ripening. The fruits of the second and third pickings (series B and C) were somewhat larger and only eighteen representative pears were taken for each sample lot. Thus the fruit of each picking was divided into twenty representative sample lots of eighteen or twenty pears each. All of the sixty samples thus prepared were weighed and placed in a ripening room in which the temperature was maintained at 67° F.

Three sample lots, one of each series, were removed from the ripening room on each of 20 successive days. Thus three series of samples were obtained and each series represented all stages of ripeness, ranging from the green condition to fruit of good eating quality and continuing on to overripeness and "internal breakdown" owing to natural causes. It was planned to obtain samples which would yield data, not only on the sequence of chemical events involved in ripening, but also on the effects of time of picking upon the ripening process.

It was found necessary to modify the experiment somewhat, since

it was desirable to discard all sample lots which had decayed during the ripening period. Daily samples were taken for 17–20 days, depending upon the number of undecayed sample lots available in each case.

METHODS OF ANALYSIS

At the time of removal from the ripening room the fruit was again weighed carefully and the loss of weight during the period of ripening computed for each of the sample lots examined.

Following weighing, samples of each lot were taken for analysis. This was accomplished by cutting two opposite longitudinal sectors from each pear, grinding the sectors through a Russwin food chopper, and then weighing duplicate 50 gm. samples from the ground pulp. These samples were preserved by boiling with sufficient 95 per cent ethyl alcohol to obtain a final concentration of 80 to 85 per cent. The preserved samples were then set aside for chemical analysis.

At the time samples were taken for analysis an attempt was made to gauge roughly the eating quality of the fruit. While such tests were obviously inaccurate, approximate data were obtained as to the time in the sequence of sampling at which the fruit was of good eating quality.

EXTRACTION.—This was carried out with the Soxhlet apparatus. The preserved samples were transferred to a weighed filter paper folded into a paper extraction cup and the filtrate collected in a volumetric flask. The extraction cup and contents were then placed in the Soxhlet apparatus and extracted with 95 per cent ethyl alcohol for at least 24 hours and until the liquid in the upper receiver showed a negative alpha-naphthol carbohydrate test. The extract was then added to the original alcoholic filtrate and the sample made up to 500 ml. with 95 per cent ethyl alcohol. The solution thus prepared contained the alcohol soluble solids.

ALCOHOL SOLUBLE SOLIDS.—These were determined gravimetrically by pipetting an aliquot of the alcohol extract into a tared 100 ml. beaker, evaporating the alcohol on the steam bath, and finally drying in a vacuum oven at 75° C. After 65 hours' drying approximately constant weight had been reached and the residue was then weighed.

ALCOHOL INSOLUBLE RESIDUE.—The residue was determined similarly by drying the tared filter paper and contents after removal from the Soxhlet apparatus. The initial weight of the filter paper and the final weight of the filter paper and residue were both obtained by drying to constant weight at 75° C. in the vacuum oven. All weighings were made in large weighing bottles to prevent absorption of moisture by the dry filter papers and residues.

TOTAL SOLIDS.—These were calculated as the sum of the alcohol soluble solids and the alcohol insoluble residue.

SUGAR DETERMINATIONS.—Determinations were made in such a way as to yield the relative amounts of sucrose, dextrose, and levulose present in the pear. Aliquot fractions of the alcohol extracts were pipetted into 250 ml. volumetric flasks and the alcohol evaporated on the steam bath. This was readily accomplished by inserting glass tubes into the necks of the flasks and blowing a stream of air on the liquid while heat was applied from below. The alcohol evaporated readily and was replaced by two additions of distilled water, 25 ml. each, after which the volume was finally reduced to about 10 ml.

The aqueous extract thus obtained was cleared by the addition of 5 ml. of a saturated solution of neutral lead acetate, delead with a slight excess of potassium oxalate, made up to volume, and filtered at once into a dry flask. The clear solution thus obtained was then ready for the determination of reducing sugars before inversion.

A separate solution was prepared for the determination of reducing sugars after inversion. The procedure was the same as the preceding, except that the aqueous extract before clarification was made up to about 50 ml., acidified with five drops of 10 per cent acetic acid and inverted by five drops of a 1 per cent solution of invertase scales. The reaction mixture was allowed to stand overnight at room temperature and then cleared, delead, and filtered as described.

REDUCING SUGARS BEFORE AND AFTER INVERSION.—These were determined by a modification of the Official Methods (2). Reduction was carried out according to the conditions of Munson and Walker, and the Cu_2O collected on asbestos pads in Gooch crucibles. The pad and Cu_2O were then suspended in 25 ml. of hot water and

the Cu_2O dissolved by heating after adding 5 ml. of 50 per cent sulphuric acid and a slight excess of a saturated solution of potassium permanganate. The excess permanganate was then decomposed by adding a saturated solution of potassium oxalate dropwise until the color of the permanganate disappeared and the pale blue of the cupric sulphate remained. The excess of the oxalate solution was limited to one drop. After cooling, 10 ml. of a 30 per cent solution of potassium iodide was added and the liberated iodine titrated with 0.05 N sodium thiosulphate. One ml. of 0.05 N sodium thiosulphate is equivalent to 3.597 mg. of Cu_2O . The sugar equivalent to the Cu_2O was obtained from the Munson and Walker tables and was expressed both as invert sugar and as dextrose.

SUCROSE.—This was calculated from the difference in reducing sugars before and after inversion, and is expressed as sucrose.

DEXTROSE AND LEVULOSE.—These were determined by the method of LOTHROP and HOLMES (11), and the determinations were carried out on samples drawn from the same solutions that were prepared for the determination of reducing sugars before inversion.

TOTAL SUGARS.—These were calculated as the numerical sum of the determined amounts of sucrose, dextrose, and levulose.

NON-SUGAR SOLUBLE SOLIDS.—These were calculated as the difference between the alcohol soluble solids and the total sugars.

SORBITOL.—This was determined by a modification of WERDER's method (18). This type of method depends on the formation of dibenzal-sorbitol from sorbitol and benzaldehyde in the presence of strong sulphuric acid as a condensing agent. In order to obtain quantitative yields of dibenzal-sorbitol the proportions of sorbitol, benzaldehyde, and sulphuric acid must be carefully controlled. Similarly the time and temperature of condensation and the method of treatment of the precipitated dibenzal-sorbitol must be judiciously carried out if approximately theoretical yields are to be obtained.

Several quantitative methods were tried out using pure sorbitol (Pfanstiehl). The method of BLEYER, DIEMAIR, and LIX (3) was found most satisfactory. Approximately quantitative yields of dibenzal-sorbitol were obtained if the precaution was taken of neutralizing the sulphuric acid with a slight excess of sodium carbonate, bringing to a boil, and finally cooling before filtering off the dibenzal,

drying and weighing. It was found that the dibenzal-sorbitol so treated could be dried at 100° C. without decomposition and that after 10–12 hours a constant weight had been attained.

The general method and proportions of the reagents used in the determination of sorbitol were essentially those listed by BLEYER, DIEMAIR, and LIX in their modification of WERDER's original procedure, but certain further modifications were made and the procedure used in this investigation is briefly described.

About 75 ml. of the alcohol extract containing the soluble solids was pipetted into a flask and 1 gm. of Mallinkrodt activated charcoal was added. The flask was tightly corked, well shaken, and the solution filtered at once through a fluted filter paper (Whatman no. 42) into a dry flask. The filtration was rapid and within 10–15 minutes a 50 ml. aliquot of the clear filtrate was pipetted into a 100 ml. beaker and evaporated on the steam bath to a very thick syrup, which solidified on cooling.

One ml. of 50 per cent sulphuric acid (1:1) and 0.5 ml. of benzaldehyde were added and the mixture stirred well for about 5 minutes and then placed in the refrigerator (1° – 3° C.) for 19–20 hours. During this time a thick yellowish white paste was formed. Fifty ml. of cold distilled water was then added and the paste broken up with a stirring rod. After remaining in the refrigerator for two hours longer a slight excess of anhydrous sodium carbonate was added (1.5 gm.). Finally one hour later the material was removed from the refrigerator, boiled for one to two minutes, and cooled in running water. The white amorphous precipitate of dibenzal-sorbitol was then collected in a tared gooch crucible, washed thoroughly with distilled water, and weighed after drying 10–12 hours at 100° C. One hundred mg. of sorbitol is equivalent theoretically to 196.8 mg. of dibenzal-sorbitol.

This method was found to give approximately quantitative yields in the range between 60 and 250 mg. of sorbitol per determination. The results of chemical tests following this procedure are shown in table 2.

The necessity of removal of sugars before determining sorbitol has been stressed by several workers (3, 15), who have reported low yields of dibenzal-sorbitol if sugars were not removed. In this in-

TABLE 2
QUANTITATIVE DETERMINATION OF SORBITOL

SORBITOL TAKEN	WEIGHT OF DIBENZAL-SORBITOL		PERCENTAGE YIELD
	OBTAINED	THEORETICAL	
75.....	148.8	147.6	100.8
75.....	149.9	147.6	100.6
100.....	198.0	196.8	100.6
100.....	200.0	196.8	101.6
125.....	240.2	246.0	97.6
125.....	240.0	246.0	97.6
150.....	277.4	295.2	83.8
150.....	267.4	295.2	90.6
175.....	346.7	344.4	100.7
175.....	344.7	344.4	100.1
200.....	395.2	393.6	100.4
200.....	398.9	393.6	101.3
250.....	492.1	492.0	100.0
250.....	492.1	492.0	99.8

TABLE 3
EFFECTS OF SUGARS ON DETERMINATION OF SORBITOL

SORBITOL TAKEN (MG.)	SORBITOL FOUND			
	SORBITOL ALONE		SORBITOL AND SUGAR*	
	WEIGHT (MG.)	YIELD (%)	WEIGHT (MG.)	YIELD (%)
75.....	78.7	104.4	77.6	103.2
100.....	105.8	105.8	105.8	105.8
125.....	134.8	107.8	131.0	105.0
150.....	144.4	96.3	154.4	103.0
175.....	168.0	96.0	171.3	97.8
200.....	184.6	92.5	191.1	96.4

* The same amount of dextrose, sucrose, and levulose was added to each sample as was present in a 50 ml. aliquot of the extract of sample B-6.

vestigation, however, it was found that if condensation were allowed to proceed for 19–20 hours in the refrigerator at 1° – 3° C. approximately quantitative yields of the dibenzal derivative were obtained from the analysis of solutions of pure sorbitol to which definite amounts of sugar had been added. The results of such tests are shown in table 3. The amounts of dextrose, levulose, and sucrose added to each sample were approximately the same as had been determined to be present in 50 ml. aliquots of pear extracts.

It may be seen from table 3 that the method used in this investigation is approximately quantitative and that errors did not exceed 8 per cent of correct values. Tests with much larger amounts of dextrose added alone to pure sorbitol showed less effect on the determination of sorbitol than shown in table 3.

Presentation of data

LOSS OF WEIGHT DURING RIPENING

The relative weight losses of pears of the three different pickings are shown in table 4 and in figure 1. These losses are expressed as percentages of the original weight of the fruit at the time it was placed in the ripening room, and represent the sum of the water loss, the carbon dioxide loss, and the loss due to other volatile products evolved by the fruit during metabolism.

It may be noted that the fruit of series B, picked September 13, lost weight somewhat more rapidly than did the fruit of series A and C which were picked on September 3 and 23 respectively. The magnitude of the weight loss was about 0.5 per cent per day in the fruit of series A and C, which corresponds approximately to the weight losses reported by HARTMAN (8) in his investigation of Bartlett pears held at 66° F. In over 400 hours there was no apparent reduction of the rate of weight loss, except perhaps in series B where a larger percentage of the original weight had been lost.

COMPOSITION OF PEARS AT VARIOUS STAGES OF RIPENING

The data presented in table 5 show the changes in the concentration of sugars and other constituents of the fruit of the three pickings as measured by the composition of individual sample lots

taken at daily intervals during the ripening period. These data are expressed as percentages of the fresh weight of the fruit at the time of withdrawal from the ripening room and thus give a measure of the composition of the fruit in so far as determined in this investigation.

TABLE 4

EFFECT OF TIME OF PICKING ON LOSS OF WEIGHT DURING RIPENING

SERIES A SEPTEMBER 3, 1935			SERIES B SEPTEMBER 13, 1935			SERIES C SEPTEMBER 23, 1935		
HOURS RIPENED	LOSS OF WEIGHT (%)	PART OF ORIGINAL WEIGHT*	HOURS RIPENED	LOSS OF WEIGHT (%)	PART OF ORIGINAL WEIGHT*	HOURS RIPENED	LOSS OF WEIGHT (%)	PART OF ORIGINAL WEIGHT*
13.....	0.36	0.9964	22	0.76	0.9924	25	0.65	0.9935
37.....	0.96	0.9904	46	1.67	0.9833	48	1.29	0.9871
61.....	1.39	0.9861	62	2.05	0.9795	70	1.36	0.9864
81.....	2.09	0.9791	94	2.95	0.9705	95	1.75	0.9825
118.....	2.86	0.9714	121	3.54	0.9646	122	2.78	0.9722
141.....	3.52	0.9648	143	4.32	0.9568	145	2.90	0.9710
166.....	3.96	0.9604	166	5.08	0.9492	169	3.24	0.9676
192.....	4.35	0.9565	192	5.91	0.9409	191	4.24	0.9576
214.....	5.05	0.9495	213	7.11	0.9289	213	4.82	0.9518
234.....	4.94	0.9506	237	7.84	0.9216	235	4.73	0.9627
260.....	5.72	0.9428	263	8.33	0.9167	265	5.39	0.9461
285.....	6.38	0.9362	286	8.18	0.9182	287	5.55	0.9445
308.....	7.04	0.9296	309	8.59	0.9141	309	6.85	0.9315
325.....	7.15	0.9285	331	9.48	0.9052	333	7.44	0.9256
360.....	7.83	0.9217	360	10.09	0.8991	361	7.84	0.9216
384.....	8.40	0.9154	384	11.81	0.8819	408		
405.....			404	11.02	0.8894	457	9.89	0.9011
432.....	10.10	0.8990						
480.....	10.83	0.8917						

* Part or proportion of original weight remaining.

The data obtained from the three different sets of fruit, picked ten days apart, show very similar composition and exhibit the same trends during the ripening process. Owing to this similarity only the data of series B are presented (fig. 2).

It may be seen from figure 2 that there was a definite increase in the total sugar content of the fruit as it ripened and that this increase continued throughout the whole period observed. The concentration of sucrose increased for the first six days of ripening and then decreased rapidly and continually for the remainder of the

period. The concentration of dextrose remained approximately constant until the decrease in sucrose was observed, and then increased as the amount of sucrose became less. Levulose increased in concentration steadily during the whole of the ripening process.

Fruit of good eating quality was obtained between the sixth and tenth day. Thereafter all fruit was inferior and internal breakdown became increasingly widespread from the eleventh day to the end of the period.

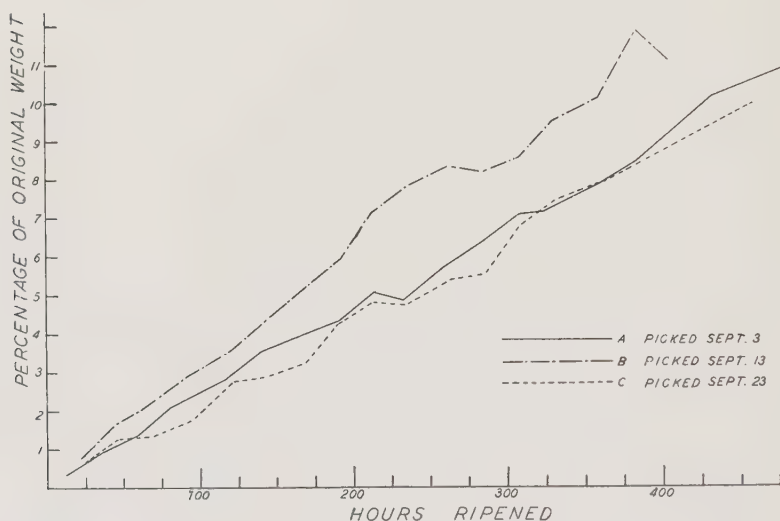


FIG. 1.—Weight loss during ripening of Bosc pears.

There were considerable fluctuations in the observed values of total solids and these fluctuations caused a marked scatter in the points upon the total solids curve as shown in figure 2.

The interpretation of data expressed in terms of fresh weight at the time of sampling may lead to erroneous conclusions, as will be shown later. The data shown in figure 2 and table 5 are presented, however, since they represent a measure of the actual composition, and since most of the American work on the physiology of fruit ripening has been expressed on this basis.

TABLE 5
COMPOSITION OF BOSCH PEARS EXPRESSED AS PERCENTAGE
FRESH WEIGHT AT TIME OF SAMPLING

DAYS RIPENED	DEX- TROSE	LEVU- LOSE	SU- CROSE	TOTAL SUGARS	NON- SUGAR SOLUBLE SOLIDS	SORBI- TOL	SOLUBLE SOLIDS	ALCOHOL INS. RES.	TOTAL SOLIDS
Series A, picked September 3, 1935									
1.....	1.95	5.47	1.93	9.35	4.54	3.73	13.89	4.30	18.19
2.....	1.64	5.76	2.38	9.78	4.31	3.54	14.09	4.04	18.13
3.....	1.95	5.47	2.08	9.50	3.99	3.18	13.49	3.94	17.43
4.....	1.99	5.74	2.67	10.40	4.26	3.08	14.66	4.13	18.79
5.....	1.76	6.06	2.62	10.44	3.46	2.87	13.90	3.81	17.71
6.....	1.70	6.05	2.60	10.35	3.25	2.61	13.60	3.62	17.22
7.....	2.09	6.16	2.02	10.27	3.54	2.56	13.81	3.67	17.48
8.....	2.20	6.11	1.99	10.30	3.36	2.39	13.66	3.87	17.53
9.....	2.29	6.61	2.29	11.19	2.96	2.33	14.15	3.90	18.05
10.....	2.30	6.69	2.19	11.18	2.81	2.23	13.99	3.68	17.67
11.....			1.62			2.17	13.53	3.41	16.94
12.....	2.40	6.71	1.48	10.59	2.59	2.09	13.18	3.77	16.95
13.....	2.57	6.88	1.08	10.53	2.78		13.31	3.56	16.87
14.....	2.63	7.36	1.00	10.99	3.06	1.98	14.05	3.52	17.57
15.....	2.72	7.31	.86	10.89	2.90		13.79	3.79	17.58
16.....	2.79	7.16	.81	10.76	2.93	1.89	13.69	3.69	17.38
17.....	2.86	7.54	.81	11.21	2.97		14.18	3.74	17.92
18.....	2.97	7.76	.71	11.44	3.00	1.98	14.44	3.96	18.40
20.....	2.93	7.56	.66	11.15	2.87	1.93	14.02	3.88	17.90
Series B, picked September 13, 1935									
1.....	1.68	5.06	2.94	9.68	4.23	3.31	13.91	3.31	17.56
2.....	1.67	5.25	3.12	10.04	4.25	3.24	14.29	3.79	18.08
3.....	1.60	5.08	3.51	10.28	3.80	2.99	14.08	3.84	17.92
4.....	1.68	5.56	3.70	10.94	3.82	2.88	14.76	3.71	18.47
5.....	1.66	5.59	3.79	11.04	3.77	2.71	14.81	3.60	18.41
6.....	1.71	5.76	3.90	11.37	3.29	2.59	14.66	3.89	18.55
7.....	1.85	5.90	3.77	11.52	3.31	2.32	14.83	3.77	18.60
8.....	1.88	6.22	3.37	11.47	3.20	2.11	14.67	3.73	18.40
9.....	1.92	6.38	3.68	11.98	3.14	2.21	15.12	3.63	18.75
10.....	2.11	6.45	2.94	11.50	3.17	2.15	14.67	3.88	18.55
11.....	2.24	6.88	3.18	12.30	3.23	2.17	15.53	3.67	19.20
12.....	2.57	6.97	2.54	12.08	3.10	2.18	15.18	3.90	19.08
13.....	2.60	6.98	2.22	11.80	2.99	1.94	14.79	3.81	18.60
14.....	2.75	7.04	2.00	11.79	2.88	1.95	14.67	3.86	18.53
15.....	2.96	7.12	1.90	11.98	3.10	1.97	15.08	3.97	19.05
16.....	3.04	7.65	1.71	12.40	2.99	1.98	15.39	3.91	19.30
17.....	3.28	7.72	1.68	12.68	2.90		15.58	3.68	19.26

TABLE 5—*Continued*

DAYS RIPENED	DEX- TROSE	LEVU- LOSE	SU- CROSE	TOTAL SUGARS	NON- SUGAR SOLUBLE SOLIDS	SORBI- TOL	SOLUBLE SOLIDS	ALCOHOL INS. RES.	TOTAL SOLIDS
Series C, picked September 23, 1935									
3.....	1.60	5.26	4.15	11.01	3.98	2.90	14.99	3.41	18.40
4.....	1.48	5.49	4.47	11.44	4.03	3.07	15.47	3.36	18.83
5.....	1.54	5.46	4.31	11.31	3.53	2.58	14.84	3.37	18.21
6.....	1.55	5.78	4.37	11.70	3.52	2.41	15.22	3.35	18.57
7.....	1.55	5.89	4.74	12.18	3.51	2.46	15.69	3.42	19.11
8.....	1.76	6.17	4.13	12.06	3.36	2.12	15.42	3.31	18.73
9.....	1.89	6.41	4.10	12.40	3.42	2.19	15.82	3.28	19.10
10.....	2.03	6.58	3.72	12.33	3.37		15.70	3.45	19.15
11.....	2.33	6.63	3.21	12.17	3.45	2.08	15.62	3.22	18.84
12.....	2.49	7.03	2.70	12.22	3.12	2.05	15.34	3.24	18.58
13.....	2.80	6.80	2.94	12.54	2.80	1.90	15.34	3.35	18.69
14.....	2.84	7.48	2.62	12.94	2.98	1.91	15.92	3.36	19.28
15.....	2.91	7.45	2.20	12.56	2.93	1.93	15.49	3.31	18.80
17.....	3.23	7.94	1.39	12.56	2.60	1.94	15.16	3.40	18.56
19.....	3.43	8.00	1.23	12.66	2.80	1.85	15.46	3.57	19.03

CHEMICAL CHANGES TAKING PLACE DURING RIPENING

The results presented in the preceding section were listed as percentages of the fresh weight at the time the samples were removed from the ripening room. In a quantitative study of chemical changes in apples, HAYNES and ARCHBOLD (10) have shown that results expressed in terms of fresh weight at the time of sampling do not necessarily show the actual changes in the various constituents of the fruit, and that, if a correct picture of the metabolic process is to be obtained, results should be expressed in terms of original fresh weight.

It was shown earlier in this paper that marked losses of weight took place as the fruit ripened. These losses amounted to about 0.5 per cent of the original fresh weight per day and were probably largely water. It might be expected that the concentration of solutes in the cell sap would increase as evaporation of water took place. By expressing results as percentages of fresh weight an increase in sugars might be observed, although no actual synthesis had taken place and the sugar already present merely had come to represent an increasing proportion of the fresh weight of the fruit.

In series B, in which the amounts of total sugars definitely increased when expressed as percentages of fresh weight at the time

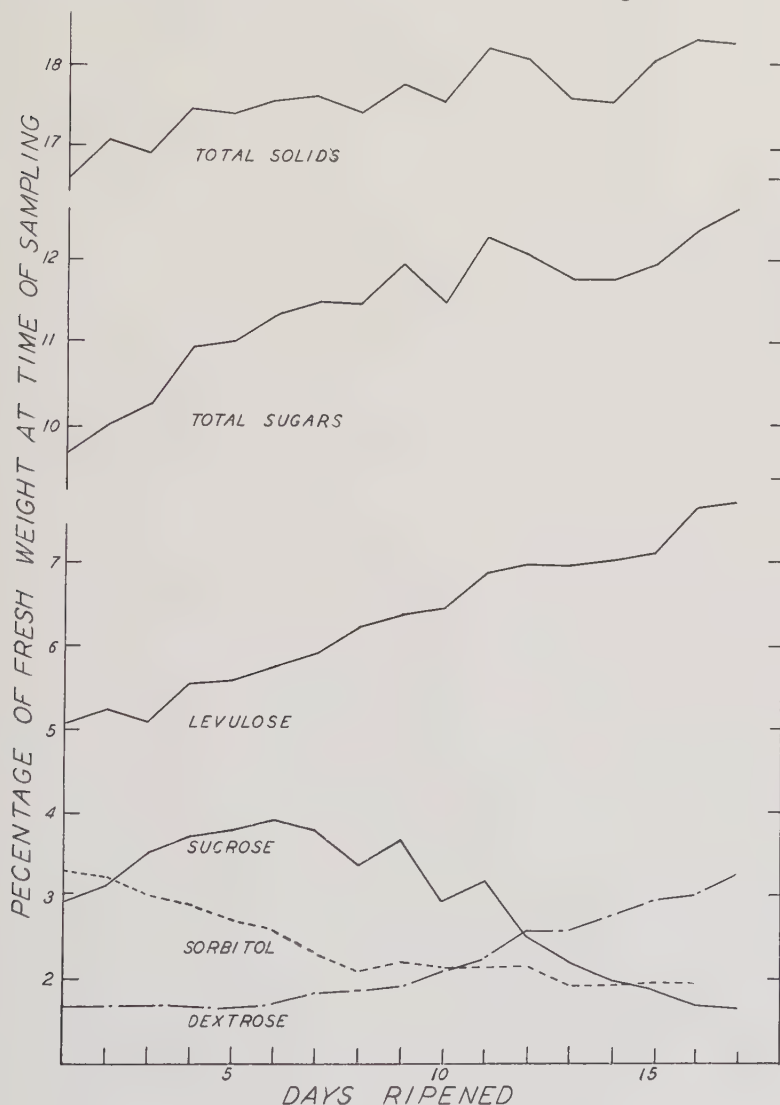


FIG. 2.—Changes in composition of Bosc pears during ripening.

of sampling, an apparent increase in total solids was also observed, as may be seen from figure 2. It seems unlikely, however, that the

amount of solid material in a pear should increase appreciably during ripening, especially in view of the fact that there should be decreases in total solids owing to losses of carbon dioxide and other volatile products of the metabolism of the fruit.

This apparent discrepancy may be explained if we recall that 11 per cent of the weight of the fruit was lost, leaving only 89 per cent of the original weight of the sample after seventeen days of ripening. Thus a 50 gm. sample taken after seventeen days represented a considerably larger proportion of the fruit than did a 50 gm. sample at the beginning of the tests. If a 100 gm. fruit were analyzed, the 50 gm. sample taken at the start would represent one half of the fruit, but after seventeen days would represent $\frac{50}{89}$ of the fruit. If results were expressed only in terms of fresh weight a comparison might be made between 100 gm. of original fruit and tissue equivalent to 112 gm. when analysis was made seventeen days later. To correct for this error in the effective size of sample, the results may be multiplied by 0.89, which is the value of the proportion of original fresh weight remaining after seventeen days of ripening. After carrying out this calculation it is found that total solids at the start of ripening represented 17.43 per cent of the original fresh weight, while after seventeen days the total solids were only 17.14 per cent of the original fresh weight of the fruit.

Thus there was actually a slight decrease in the total solid material during ripening, whereas the calculation of results in terms of fresh weight at time of sampling showed an increase in total solids.

In order to ascertain the actual changes in the various constituents of the fruit as ripening took place, the data shown in table 5 were recalculated as percentages of the original fresh weight. This was accomplished by multiplying the values listed in the table by the corresponding values of "proportion of original weight remaining" which are listed in table 4. By these calculations all data were reduced to a comparable basis, percentages of original fresh weight, and have been so listed in table 6. As before, only the data of series B are presented (fig. 3), since the changes shown by the fruit of this series appear to be representative of all three sets studied.

CARBOHYDRATE CHANGES IN RIPENING.—The data plotted in figure 3 show a definite increase in levulose throughout the whole

TABLE 6
CHANGES IN CONSTITUENTS OF BOSCH PEARS EXPRESSED AS
PERCENTAGE ORIGINAL FRESH WEIGHT

DAYS RIPENED	DEX- TROSE	LEVU- LOSE	SU- CROSE	TOTAL SUGARS	NON- SUGAR SOLUBLE SOLIDS	SORBI- TOL	TOTAL SOLUBLE SOLIDS	ALCOHOL INS. RES.	TOTAL SOLIDS
Series A, picked September 3, 1935									
1.....	1.94	5.45	1.93	9.32	4.52	3.72	13.84	4.28	18.12
2.....	1.62	5.70	2.36	9.68	4.27	3.51	13.95	4.00	17.95
3.....	1.92	5.39	2.06	9.37	3.93	3.14	13.30	3.88	17.18
4.....	1.95	5.62	2.61	10.18	4.17	3.02	14.35	4.05	18.40
5.....	1.71	5.88	2.55	10.14	3.36	2.79	13.50	3.70	17.20
6.....	1.64	5.84	2.51	9.99	3.13	2.52	13.12	3.49	16.61
7.....	2.01	5.92	1.94	9.87	3.40	2.46	13.27	3.52	16.79
8.....	2.10	5.85	1.90	9.85	3.22	2.29	13.07	3.70	16.77
9.....	2.17	6.28	2.17	10.62	2.81	2.21	13.43	3.70	17.13
10.....	2.19	6.36	2.08	10.63	2.67	2.12	13.30	3.50	16.80
11.....			1.53			2.05	12.76	3.21	15.97
12.....	2.25	6.28	1.39	9.92	2.42	1.96	12.34	3.53	15.87
13.....	2.40	6.40	1.00	9.80	2.58		12.38	3.30	15.68
14.....	2.44	6.83	.93	10.20	2.84	1.84	13.04	3.27	16.31
15.....	2.51	6.74	.79	10.04	2.67		12.71	3.49	16.20
16.....	2.55	6.55	.75	9.85	2.68	1.73	12.53	3.38	15.91
18.....	2.67	6.98	.64	10.29	2.70	1.78	12.99	3.55	16.54
20.....	2.61	6.74	.59	9.94	2.56	1.72	12.50	3.46	15.96
Series B, picked September 13, 1935									
1.....	1.67	5.02	2.92	9.61	4.20	3.29	13.80	3.62	17.42
2.....	1.64	5.16	3.07	9.87	4.18	3.19	14.05	3.73	17.78
3.....	1.65	4.98	3.44	10.07	3.72	2.93	13.79	3.76	17.55
4.....	1.63	5.40	3.59	10.62	3.71	2.80	14.33	3.60	17.93
5.....	1.60	5.39	3.66	10.65	3.64	2.61	14.29	3.47	17.76
6.....	1.64	5.51	3.73	10.88	3.15	2.48	14.03	3.72	17.75
7.....	1.76	5.60	3.58	10.94	3.14	2.20	14.08	3.58	17.66
8.....	1.77	5.85	3.17	10.79	3.01	1.98	13.80	3.51	17.31
9.....	1.78	5.93	3.42	11.13	2.92	2.05	14.05	3.37	17.42
10.....	1.94	5.94	2.72	10.60	2.92	1.98	13.52	3.58	17.10
11.....	2.05	6.31	2.92	11.28	2.96	1.99	14.24	3.36	17.60
12.....	2.36	6.40	2.33	11.09	2.85	2.00	13.94	3.58	17.52
13.....	2.38	6.38	2.03	10.79	2.73	1.77	13.52	3.48	17.00
14.....	2.49	6.37	1.81	10.67	2.61	1.77	13.28	3.49	16.77
15.....	2.66	6.40	1.71	10.77	2.79	1.77	13.56	3.57	17.13
16.....	2.68	6.74	1.51	10.93	2.64	1.75	13.57	3.45	17.02
17.....	2.92	6.87	1.49	11.28	2.58		13.86	3.27	17.13

TABLE 6—Continued

DAYS RIPENED	DEX- TROSE	LEVU- LOSE	SU- CROSE	TOTAL SUGARS	NON- SUGAR SOLUBLE SOLIDS	SORBI- TOL	TOTAL SOLUBLE SOLIDS	ALCOHOL INS. RES.	TOTAL SOLIDS
Series C, picked September 23, 1935									
3.....	1.58	5.19	4.09	10.86	3.93	2.86	14.79	3.36	18.15
4.....	1.46	5.39	4.39	11.24	3.96	3.02	15.20	3.30	18.50
5.....	1.50	5.31	4.19	11.00	3.43	2.51	14.43	3.28	17.70
6.....	1.51	5.61	4.24	11.36	3.42	2.34	14.78	3.25	18.03
7.....	1.50	5.70	4.59	11.79	3.40	2.38	15.19	3.30	18.49
8.....	1.69	5.91	3.95	11.55	3.22	2.03	14.77	3.17	17.94
9.....	1.80	6.10	3.90	11.80	3.26	2.08	15.06	3.12	18.18
10.....	1.93	6.27	3.54	11.74	3.21		14.95	3.29	18.24
11.....	2.20	6.27	3.04	11.51	3.26	1.97	14.77	3.05	17.82
12.....	2.35	6.64	2.55	11.54	2.95	1.94	14.49	3.06	17.55
13.....	2.61	6.33	2.74	11.68	2.61	1.77	14.29	3.12	17.41
14.....	2.63	6.92	2.43	11.98	2.76	1.77	14.74	3.11	17.85
15.....	2.68	6.87	2.03	11.58	2.70	1.78	14.28	3.05	17.33
19.....	3.09	7.21	1.11	11.31	2.52	1.67	13.93	3.22	17.15

of the ripening process. Sucrose increased during the first six days and then decreased steadily for the remainder of the period. While sucrose was increasing, dextrose remained constant; but as sucrose was hydrolyzed, dextrose appeared to be formed. As might be expected, the increases in dextrose and levulose accompanying the decrease in sucrose were approximately equal.

The points on the graphs show considerable scatter and it is difficult to judge the exact slope of the curves in some cases. The general trends may be observed, however, and a fair approximation made of the magnitude of the changes in the several sugars present.

Total sugars were found to increase definitely for the first six days; beyond that point little change could be observed with certainty owing to the scatter of the points on the curve. Total solids decreased during the ripening process. As has already been pointed out, this finding is more understandable than that observed when results were calculated as percentages of the fresh weight at the time of sampling.

The data for series A and C, the early and late picked fruit respectively, show essentially the same results as have been described,

except that the increases in total sugars were less marked and the decreases in total solids somewhat greater.

The early picked fruit contained less sucrose at all stages of ripe-

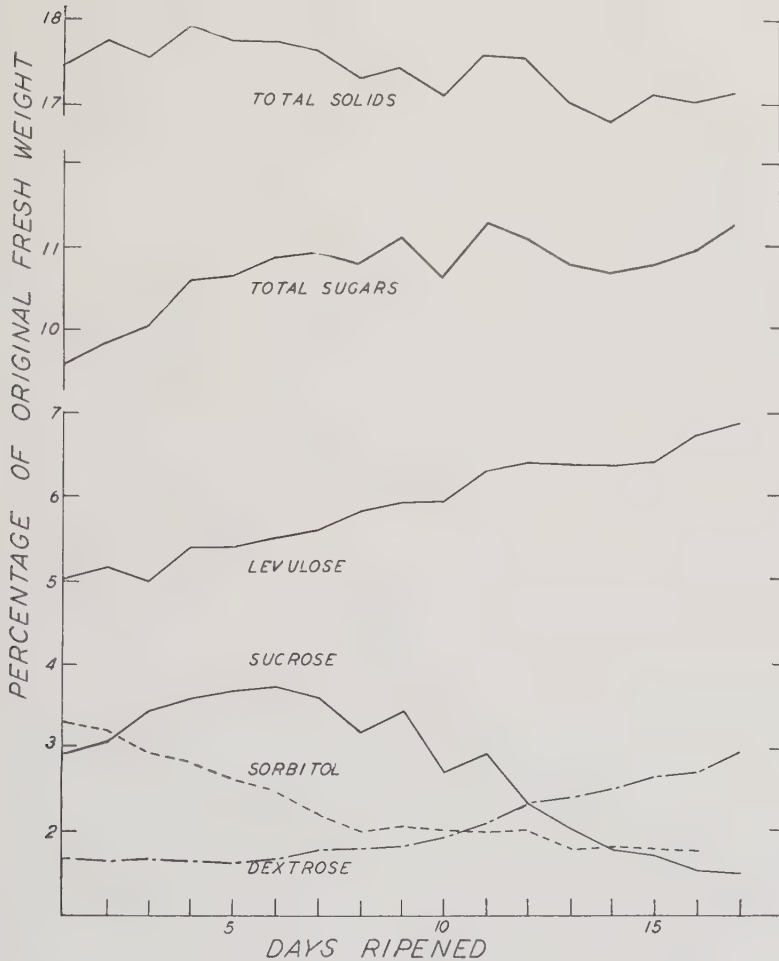


FIG. 3.—Changes which take place in the constituents of Bosc pears during ripening.

ness than did the fruit of the commercial picking. On the other hand, the late picked fruit showed more sucrose throughout the whole period than did the fruit picked at the time of commercial

harvest. A comparison of the relative sucrose content and its fluctuation during ripening is shown in figure 4. The dextrose and levulose contents of fruit of the three pickings were about the same and differences in total sugars in fruit of the three pickings were caused by the differences in sucrose.

Starch might be expected to serve as the material from which sugars were formed during the ripening process. Microchemical tests, however, indicated that only slight traces of starch were present. Moreover, the alcohol insoluble residue which would have con-

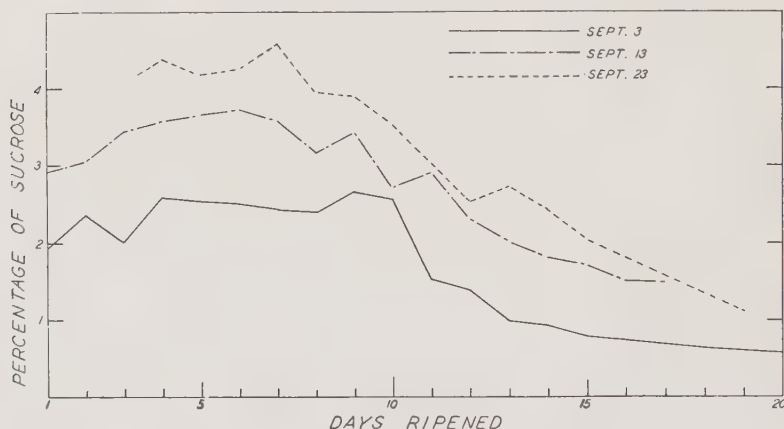


FIG. 4.—Effect of time of picking on sucrose changes during ripening.

tained the starch, if present, showed only very small decreases during the ripening of the fruit of series B and C, and only slight decreases in the fruit of series A. The magnitude of the observed decreases of this residue were found to be insufficient to account for the amounts of sugar observed to be formed during the ripening of the fruit. Starch determinations on the alcohol insoluble residues of the initial members of series A, B, and C showed 0.20, 0.20, and 0.28 per cent of starch respectively.

SORBITOL CHANGES IN RIPENING.—It was noticed from the data presented in the preceding section that the values of total sugars differed markedly from the corresponding values of soluble solids. This discrepancy was found to amount to 2.6–4.5 per cent of the fresh weight of the fruit, and suggested the presence of some major

constituent of a soluble non-sugar nature. The values of the differences between soluble solids and total sugars were calculated and designated non-sugar soluble solids. It was found that the values of this unknown undetermined constituent of the fruit decreased definitely during the ripening process and were roughly equal to or slightly in excess of the observed increases in total sugars. The values of this fraction of the fruit are shown in tables 5 and 6.

EMMETT (5) has reported that in the analysis of Conference pears there was a similar discrepancy between soluble solids and the sum of the separately determined constituents of the fruit. He calculated that 2 to 3 per cent of the fresh weight of the pear pulp was unidentified. Further, he noted that the amounts of this unidentified constituent decreased during storage, and suggested that the substance in question may have been sorbitol. He made no determinations, however, to substantiate this suggestion.

The presence of sorbitol in pears has been reported by VINCENT and DELACHANAL (17) and by REIF (15). Recently NUCCORINI and BARTOLI (14) have found that the sorbitol content of fruits of *Sorbus domestica* decreases when the fruit is detached from the tree, and they state that it is transformed into dextrose and levulose.

In view of these findings it was decided to determine qualitatively whether or not sorbitol occurred among the soluble solids of the Bosc pear. Certain of the alcohol extracts were evaporated to a thick syrup and this syrup allowed to react with benzaldehyde in the presence of sulphuric acid as a condensing agent. After standing overnight in the refrigerator, a white amorphous precipitate was obtained. This was then washed free of benzaldehyde and sulphuric acid. It was found to melt at 174° – 177° C. and appeared to be dibenzal-sorbitol. DAVIS, SLATER, and SMITH (4) report that dibenzal-sorbitol prepared by this method melts at 179° – 184° C. Owing to the difficulty of purifying this compound no recrystallization was carried out.

In order to demonstrate that the benzaldehyde derivative so obtained was definitely derived from sorbitol, a quantity of the material was hydrolyzed and then acetylated according to the methods given by TUTIN (16). The acetate so obtained was recrystallized from ethyl ether, and hard colorless crystals were ob-

tained. These crystals melted at 98° – 99° C., which is the range reported by DAVIS, SLATER, and SMITH and by TUTIN for sorbitol hexaacetate. The acetate was also prepared by the same method from pure sorbitol; its melting point and appearance were found to be identical with that derived from pear extracts as just described.

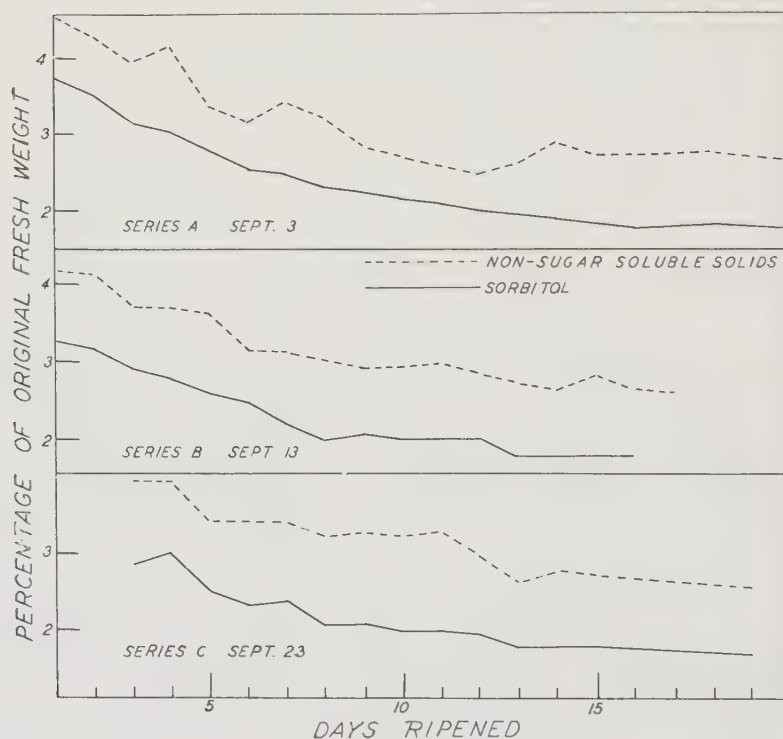


FIG. 5.—Relation of sorbitol to non-sugar soluble solids.

From the evidence presented it was assumed that sorbitol was definitely a constituent of the Bosc pear. Quantitative measurements were then made using the method outlined in the section on methods. The changes in sorbitol during ripening are listed in table 6 and shown graphically in figures 3 and 5. The changes in sorbitol (fig. 5) are plotted together with the corresponding changes in the

hitherto unidentified fraction which was designated as the non-sugar soluble solids.

The data shown in figure 5 indicate that sorbitol definitely decreases during the ripening process. In the fruit of all three pickings the decreases in the non-sugar soluble solids were accompanied by corresponding decreases in sorbitol. The most rapid decreases in sorbitol were found to take place in the first six to eight days, when both sucrose and levulose were observed to be formed from some hitherto unknown source.

The fact that the curves for sorbitol (fig. 5) follow the curves for non-sugar soluble solids indicates that the undetermined fraction contained sorbitol and that as sorbitol decreased the value of this fraction decreased. After subtracting sorbitol from the non-sugar soluble solids there still remained a value of 0.6 to 1.0 per cent, which seems reasonable for the sum of organic acids, soluble nitrogen, and other minor soluble constituents.

Discussion

In previous reports on the chemical changes in the ripening of pears, the duration of ripening has been taken as the time required to obtain fruit of good eating quality. In this investigation the results include data on the period following the attainment of optimum eating quality and continue to the time of occurrence of severe internal breakdown. The period studied, according to the usual criteria, might be said to include both ripening and over-ripening. In comparing the data reported here with those of other published reports, therefore, comparable data must be taken.

The ripening process, from the point of view of sugar changes, may be considered to consist of two definite stages: the first in which sucrose is formed, and the second in which sucrose is hydrolyzed with corresponding increases in dextrose and levulose. The attainment of fruit of good eating quality appears to occur in the four days following the beginning of hydrolysis of sucrose.

The essential differences in the sugar content of ripe and over-ripe Bosc pears appear to be the relative amounts of sucrose and reducing sugars. Total sugars show no decrease with over ripening, but the

amounts of reducing sugars are far greater and the amounts of sucrose far less than in fruit of good eating quality. The composition of sound and over-ripe Conference pears in storage has been determined by EMMETT (5), and his results show higher values of sucrose in sound than in over-ripe fruit, together with larger amounts of reducing sugars in over-ripe than in sound fruit. The results of the present investigation are in agreement with his.

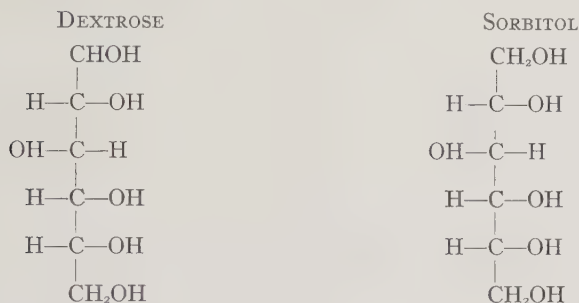
MAGNESS (12) and EZELL and DIEHL (6) have shown that in the ripening of Bartlett pears reducing sugars increased during ripening, and that in nearly all cases sucrose increased as well. If the composition of green Bosc pears is compared with that of ripe pears of good eating quality, essentially the same results may be observed, since during the first phase of ripening definite increases in the concentration of both sucrose and levulose were found.

MAGNESS has noted a much higher sugar content in fruit ripened at 70° F. than in comparable fruit ripened at lower temperatures. EMMETT (5), however, has suggested that the high sugar content observed by MAGNESS in fruit ripened at 70° F. may have been caused by a larger water loss by transpiration, for which no correction was made in the calculation of the sugar content. MAGNESS explained the larger increase in sugars at 70° F. as apparently due either to a smaller loss of sugar in respiration or to the fact that more insoluble or non-reducing substances in the green fruit are changed to soluble reducing material when the fruit is ripened at the optimum temperature.

The results presented in the preceding sections show the necessity of correcting for weight losses in ripening, but also indicate that even after such corrections are made, there are still definite increases in sugars which cannot be accounted for by decreases in the insoluble material of the fruit. The observed increases in sugars can easily be accounted for by the observed decreases in sorbitol. The presence of sorbitol in pears has been previously reported, but evidence for its transformation to sugars has not heretofore been presented.

Sorbitol may readily be oxidized to glucose chemically, and the manufacture of sorbitol is at present carried out by the reduction of glucose. It does not seem unlikely, therefore, that sorbitol could be oxidized to sugar in the processes of metabolism. The relationship

between the sugar alcohol, sorbitol, and dextrose may be seen from the following formulae of the two substances:



From the results presented here it appears that levulose and sucrose, rather than dextrose, are the sugars formed from sorbitol. During the most rapid decrease in sorbitol practically no dextrose was observed to be formed.

In the fruit of series B the transformation of sorbitol to sugars was almost quantitative, while in series A and C the decreases of sorbitol were considerably larger than the observed increases in sugars. In the latter two cases considerable losses in dry matter were noted, however, and it may be suggested that losses of sugar in respiration were sufficiently great that a smaller net gain in sugar was observed than would be expected from a quantitative oxidation of sorbitol to sugar.

NUCCORINI and BARTOLI have found that sorbitol is transformed to dextrose and levulose in the ripening of the fruits of *Sorbus domestica*. Such a transformation in the pear, accompanied by a preferential oxidation of dextrose in respiration, would lead to an excess of levulose over dextrose in the fruit. If the remaining dextrose combined with levulose to form sucrose and the excess levulose remained unchanged, we would observe in effect a transformation of sorbitol to sucrose and levulose, as appears to take place in the ripening of Bosc pears.

Whatever may be the mechanism of sorbitol metabolism in pears, it does definitely disappear in the ripening process. DAVIS, SLATER, and SMITH have found the heat of combustion of sorbitol in solution to be 732.44 kilogram calories per mol as compared with 676.08 for

dextrose. Thus an oxidation of a mol of sorbitol to glucose would release 66.36 kilogram calories of energy. The transformation of sorbitol to sugar by oxidation may therefore provide some of the energy for the metabolism of the fruit without loss of carbon dioxide, and thus represent a type of aerobic respiration which would not be recorded by the usual methods of measuring respiration.

Summary

1. A study of the ripening process of Bosc pears held at 67° F. has been made by means of chemical analyses of individual sample lots removed from the ripening room on each of 17-20 successive days. Pears of three different lots, picked ten days apart, were studied in this way and determinations made of total solids, alcohol insoluble residue, soluble solids, dextrose, levulose, sucrose, and sorbitol.

2. The losses of weight during the ripening period were recorded. The results of sugar and other determinations were calculated in terms of fresh weight at the time of sampling as well as in percentages of the original fresh weight. The latter method, involving correction for changes in effective size of sample taken, appears to give a more accurate method of studying the changes taking place during ripening.

3. A study of the sugar changes during ripening showed that levulose increased during the entire ripening period. Sucrose increased for the first six to eight days and then decreased throughout the rest of the period studied. Dextrose remained constant during the formation of sucrose, but increased later as sucrose disappeared. The increases in dextrose and levulose accompanying the decrease in sucrose were approximately equal, and their sum was about the same amount as that of the sucrose hydrolyzed.

4. The principal difference in the sugar content of fruit of the three pickings lay in the differences in the amounts of sucrose present. Throughout the ripening period the early picked fruit showed the least sucrose, the fruit of the commercial picking an intermediate amount, and the late picked fruit the highest values of this sugar.

5. Total sugars, calculated as the sum of dextrose, levulose, and sucrose, increased during the ripening process; this increase was

considerably greater than could be accounted for by the observed decreases in the alcohol insoluble residue.

6. Sorbitol was identified as a constituent of Bosc pears, and a quantitative method was devised for its measurement. Sorbitol was observed to decrease during the ripening process in fruit of all three pickings, and these decreases were large enough to account for the observed increases in total sugars.

The writer wishes to acknowledge his appreciation for the encouragement and helpful suggestions offered by Professors C. A. SHULL and S. V. EATON during the course of this investigation.

UNIVERSITY OF CHICAGO

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